Notes

Microbiological Oxygenation of 1-Azidoadamantane and of *N*-Benzoyl-3-noradamantanamine

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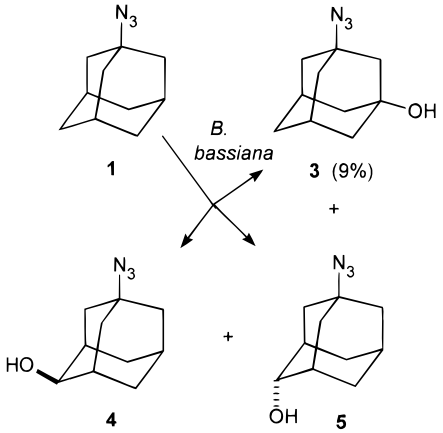
In 1967−1971, we reported a series of experiments that explored the capability of microorganisms to oxygenate a variety of acyclic, monocyclic, bicyclic, and polycyclic molecules.[2] The fungus, *Beauveria bassiana*,[3] was found to be very good at performing the oxygenation of a broad range of such molecules. Among the classes of compounds that we studied at that time were the 1-amidoadamantanes;[4] these studies were extended to include the asymmetric oxygenation of 4-methyl-*N*-benzoyl-1-aminoadamantane[5] and the oxygenation of 2-benzamidoadamantane[6] using *B. Bassiana*. Other groups have reported the oxygenation of diamantanols with *Rhizopus nigricans*,[7] of various acyl derivatives of 1-aminoadamantane using *B. bassiana*,[8] and of adamantyl carbamates with *B. bassiana*.[9]

In this paper, we describe the oxygenation of two additional adamantane or adamantane-like compounds using *B. bassiana*. The first is the oxygenation of 1-azidoadamantane (**1**), which, to our knowledge, is the first report of the microbiological oxygenation of a simple organic azide. The second substrate studied is *N*-benzoyl-3-noradamantanamine (**2**), which is structurally related to the 1-amidoadamantanes of our earlier report.[4] In contrast to a 1-amidoadamantane, however, the noradamantane nucleus of **2** presents an interesting potential substrate since it contains 10 prochiral hydrogens and only three nonprochiral hydrogens.

Oxygenation of 1-Azidoadamantane (1). Bioconversion of **1** using *B. bassiana* gave three new compounds in a total unoptimized yield of 18%. One of the products (**3**, 9%) was separated from the other two (**4** and **5**, 9% combined) by chromatography and was characterized by ^{13}C NMR data as the tertiary alcohol of structure **3**. A



signal at δ 69.6 was assigned to the carbinol carbon, and this carbon was shown by a ^{13}C DEPT experiment to be quatenary; consequently, the alcohol must be tertiary. Only one structure is possible for such a tertiary alcohol and is represented by structure **3**.

The other two bioconversion products could not be separated despite extensive efforts using chromatography and, therefore, were characterized as a mixture. The two compounds were judged from the ^{1}H NMR spectrum to be secondary alcohols and were present in a ratio of 86:14 (**4**:**5**) according to integration of the spectrum. Oxidation of the mixture gave a single ketone (**6**) showing that the two alcohols have an epimeric relationship to one another. By carrying out the chemical transformations as shown below, the two isomers were correlated with compounds of known structure.

Briefly, the correlation was achieved by catalytic reduction of the mixture of **4** and **5**, which served to reduce the azide groups to amino groups (**7** and **8**). The mixture of amino alcohols **7** and **8** was acetylated, giving the bisacetyl derivatives **9** and **10**. Authentic samples of **9** and **10** were obtained by acetylation of known[4] alcohols **11** and **12**. This correlation shows that the major secondary alcohol **4** has a *cis*-configuration (with respect to the azide group) and that the minor alcohol **5** has the *trans*-configuration. The epimeric alcohols **4** and **5** may be primary hydroxylation products or may arise through a sequence of hydroxylation, dehydrogenation to a ketone (via an alcohol dehydrogenase), and enzymic reduction back to the hydroxyl groups. If they are primary hydroxylation products, the introduction of oxygen into 1-azidoadamantane clearly differs stereochemically from 1-amidoadamantanes that are hydroxylated exclusively *trans* to the amide groups.[4]

Oxygenation of *N*-Benzoyl-3-noradamantanamine (2). As noted in the introduction, the noradamantane nucleus provides more stereochemically different sites for oxygenation than does the adamantane nucleus. The availability of 3-noradamantanamine·HCl (**13**) from a commercial source provided us with an opportunity to examine microbiological oxygenation of a derivative of this compound. We prepared the benzamide **2** from the

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(2) For a summary and references to many of these results, see: Johnson, R. A. In *Oxidation in Organic Chemistry*; Trahanovsky, W. S., Ed.; Academic Press: New York, 1978; Part C, p 131.

(3) The classification of this microorganism has changed over the years; *Sporotrichum sulfurescens* was changed to *Beauveria sulfurescens* in 1970 (Taylor, J. J. *Mycologia* **1970**, *62*, 797) and currently is listed as *Beauveria bassiana* (ATCC 7159) in the catalog of the American Type Culture Collection.

(4) Herr, M. E.; Johnson, R. A.; Murray, H. C.; Reineke, L. M.; Fonken, G. S. *J. Org. Chem.* **1968**, *33*, 3201.

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(7) Blaney, F.; Johnston, D. E.; McKervey, M. A.; Jones, E. R. H.; Pragnell, J. *J. Chem. Soc., Chem. Commun.* **1974**, 297.

(8) Bailey, P. D.; Higgins, S. D.; Ridyard, C. H.; Roberts, S. M.; Rosair, G. M.; Whittaker, R. A.; Willetts, A. J. *J. Chem. Soc., Chem. Commun.* **1996**, 1833.

(9) Vigne, B.; Archelas, A.; Furstoss, R. *Tetrahedron* **1991**, *47*, 1447.

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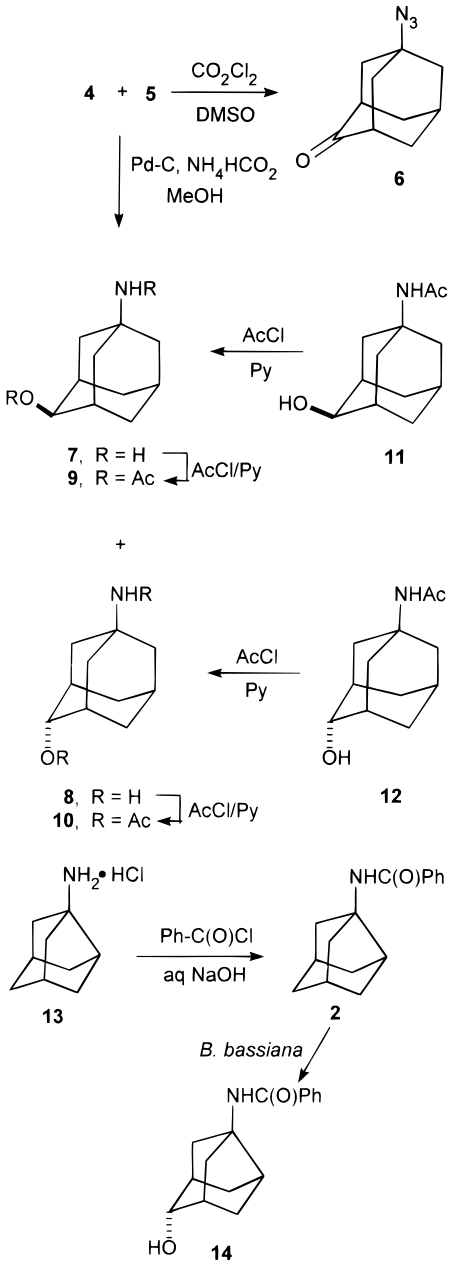

amine·HCl **13** and carried out a bioconversion with *B. bassiana*. A single product of oxygenation was isolated from the bioconversion and was obtained in a yield of 58%.

Our hope for an interesting stereochemical result was not met when we interpreted the ^{1}H and ^{13}C NMR spectra of the product. The ^{1}H spectrum showed that the new compound was a secondary alcohol, and the ^{13}C spectra (normal and DEPT) revealed that the compound was symmetrical with regard to its stereochemistry. Therefore, oxygenation must have occurred on the C-9 methylene group.

The configuration of the hydroxyl group at C-9 was determined by an X-ray crystallographic structure analysis. From this analysis, the hydroxyl group is found to be *trans* with respect to the C-3 benzamide substituent as shown in **14**. Consequently, with respect to stereochemistry, oxygenation of *N*-benzoyl-3-noradamantanamine with *B. bassiana* closely parallels the results obtained in earlier work[4] with *N*-benzoyl-*N*-methyl-1-adamantanamine.

Experimental Section

Biotransformation Process. *B. bassiana* (ATCC 7159) was used for the fermentations described herein. Fermentation conditions and procedures for isolation of products are described in ref 10.

Preparation of 1-Azidoadamantan-3-ol (3), 1-Azidoadamantan-*cis*-4-ol (4), and 1-Azidoadamantan-*trans*-4-ol (5) by Bioconversion of 1-Azidoadamantane (1) with *B. bassiana*. Bioconversion of **1** (0.50 g, 2.8 mmol) was carried out in 50 shake flasks for 3.5 days. Following workup (see general procedure in ref 10), the residue was chromatographed on SiO_2 (5% MeCN−CH_2Cl_2; **3,** $R_f = 0.30$; **4** and **5**, $R_f = 0.40$). Isomer **3** was again chromatographed on SiO_2 (5% MeCN−CH_2Cl_2) to give 0.048 g (0.25 mmol, 9%) as a white solid: mp 88 °C; ^{1}H NMR ($CDCl_3$) δ 1.53 (m, 2 H), 1.67−1.71 (m, 8 H), 1.76 (s, 2H), 2.33 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 30.8, 34.5, 40.2, 43.8, 49.1, 60.7, 69.6; IR (mull) 3360 (s), 2923 (s), 2088 (s), 1455 (s), 1351 (m), 1304 (m), 1099 (s), 996 (s) cm^{-1}. Anal. Calcd for $C_{10}H_{15}N_3O$: C, 62.15; H, 7.82; N, 21.75. Found: C, 62.86; H, 7.78; N, 20.72.

Isomers **4** and **5** were further chromatographed on SiO_2 (20% EtOAc−hexane, $R_f = 0.25$) to give a total of 0.048 g (0.25 mmol, 9%) of **4** and **5** as a 86:14 mixture. Column fractions 6−7 contained 0.025 g (88:12) and fractions 8−11 contained 0.023 g (82:18) of the **4**/**5** mixture. Fractions 6−7 (88:12) were solid: mp 85−92 °C; ^{1}H NMR ($CDCl_3$) δ 1.5−1.9 (m, 9 H), 2.0−2.2 (m, 1 H), 3.77 (t, $J = 3.0$ Hz, 0.9 H), 3.89 (t, $J = 3.0$ Hz, 0.1 H); ^{13}C NMR ($CDCl_3$) δ 28.5, 34.7, 35.1, 36.3, 41.5, 58.3, 72.3; IR (mull) 3311 (s), 2855 (s), 2951 (s), 2923 (s), 2089 (s), 1454 (m), 1256 (m), 1064 (m), 1033 (m) cm^{-1}; HRMS calcd for $C_{10}H_{15}N_3O$ 193.1215, obsd 193.1207.

Conversion of 1-Azidoadamantan-4-ol Isomers 4 and 5 to 1-Azidoadamantan-4-one (6). A solution of **4** and **5** (82:18, 0.013 g, 0.067 mmol) in CH_2Cl_2 (8 mL) was oxidized using oxalyl chloride (0.017 g, 0.134 mmol), DMSO (0.021 g, 0.27 mmol), and Et_3N (0.039 g, 0.335 mmol). After workup, chromatography on SiO_2 (5% EtOAc−hexane, $R_f = 0.15$) gave 0.010 g (0.052 mmol, 78%) of **6** as a viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.95−2.15 (m, 10 H), 2.35 (bs, 1 H), 2.65 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 29.01, 37.97, 40.43, 41.84, 46.47, 57.34, 215.02; IR (mull) 2930 (s), 2093 (s), 1721 (s), 1452 (m), 1261 (m), 1252 (m), 1063 (m), 1053 (m) cm^{-1}; HRMS calcd for $C_{10}H_{13}N_3O$ 191.1059, obsd 191.1058.

Conversion of 1-Azidoadamantan-4-ol Isomers 4 and 5 to *N,O*-Diacetyladamantanamine Isomers 9 and 10. A 10 mL flask equipped with a stir bar was charged with Pd−C (10%, 0.020 g), MeOH (5 mL), ammonium formate (0.021 g, 0.335 mmol), and a mixture of alcohols **4** and **5** (88:12, 0.013 g, 0.067 mmol). The mixture was stirred at rt for 2 h and then filtered through Celite to remove catalyst. The methanolic filtrate was concentrated to give 0.008 g of crude amino alcohol mixture (**7** and **8**). The residue was diluted with CH_2Cl_2 (5 mL) and transferred to a 15 mL flask followed by addition of pyridine (0.2 mL) and acetyl chloride (0.025 g, 0.32 mmol). The solution was stirred 15 min at rt, diluted with CH_2Cl_2 (10 mL), and washed with saturated $CuSO_4$ solution (10 mL). The CH_2Cl_2 phase was dried (Na_2SO_4) and concentrated to give a crude residue that was chromatographed on SiO_2 (20% acetone−hexane, $R_f = 0.20$) to give 0.007 g (42%) of **9** and **10**; ^{1}H and ^{13}C NMR data for the isomeric mixture correlated with that obtained on authentic samples prepared and characterized as described below.

Preparation of *N,O*-Diacetyl-1-aminoadamantan-*cis*-4-ol (9). Alcohol **11** was prepared by $NaBH_4$ reduction of the corresponding ketone as described in the literature.[4] Following the procedure described below for preparation of **10** from **12**, acetylation of **11** (0.085 g, 0.40 mmol) was carried out using CH_2Cl_2 (7 mL), pyridine (0.5 mL), and acetyl chloride (0.079 g, 1.0 mmol). Following workup, chromatography on SiO_2 (20% acetone−hexane, $R_f = 0.20$) gave 0.094 g (0.37 mmol, 92%) of **9** as a white solid: mp 151 °C; ^{1}H NMR ($CDCl_3$) δ 1.65−1.86 (m, 6 H), 1.90 (s, 3 H), 2.03 (s, 3 H), 2.07 (s, 3 H), 2.12−2.28 (m, 4 H), 4.81 (m, 1 H), 5.15 (bs, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.4, 24.6, 28.1, 33.3, 34.9, 36.0, 41.0, 51.0, 75.2, 169.3, 170.5; IR (mull) 3312 (m), 2915 (s), 1733 (s), 1650 (s), 1553 (s), 1365 (s), 1263 (s), 1247 (s), 1035 (m) cm^{-1}; HRMS calcd 251.1521, obsd 251.1523. Anal.

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Calcd for $C_{14}H_{21}NO_3$: C, 66.91; H, 8.42; N, 5.57. Found: C, 66.85; H, 8.37; N, 5.58.

Preparation of *N,O*-Diacetyl-1-aminoadamantan-*trans*-4-ol (10). Into a 25 mL flask were placed alcohol **12**[4] (0.025 g, 0.12 mmol), CH_2Cl_2 (10 mL), and pyridine (0.2 mL). Acetyl chloride (0.050 g, 0.64 mmol) was added, and the solution was stirred 15 min at rt and then washed with saturated $CuSO_4$ solution (10 mL). The CH_2Cl_2 phase was dried (Na_2SO_4) and concentrated to give a crude residue that was chromatographed on SiO_2 (20% acetone−hexane, $R_f = 0.20$) to give 0.025 g (83%) of **10** as a white solid; mp 131−132 °C; ^{1}H NMR ($CDCl_3$) δ 1.49 (m, 2 H), 1.90 (s, 3 H), 1.94−2.03 (m, 8 H), 2.07 (s, 3 H), 2.11 (bs, 3 H), 4.90 (bs, 1 H), 5.17 (bs, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.4, 24.6, 28.4, 30.51, 32.6, 40.0, 41.3, 51.0, 75.7, 169.5, 170.5; IR (mull) 2914 (s), 1740 (s), 1721 (s), 1649 (s), 1673 (s), 1541 (s), 1456 (s), 1373 (s), 1254 (s) cm^{-1}; HRMS $C_{14}H_{21}NO_3$ requires 251.1521, found 251.1523. Anal. Calcd for $C_{14}H_{21}NO_3$: C, 66.91; H, 8.42; N, 5.57. Found: C, 66.62; H, 8.65, 5.47.

Preparation of *N*-Benzoyl-3-noradamantanamine (2). A 125 mL separatory funnel containing 2 N NaOH (50 mL) and 3-noradamantanamine hydrochloride (**13**, 0.70 g, 4.0 mmol) was shaken for 1 min followed by addition of benzoyl chloride (0.90 g, 6.4 mmol). The funnel was shaken vigorously for 10 min, and then the mixture was extracted with CH_2Cl_2 (4 × 30 mL). The combined CH_2Cl_2 phases were dried (Na_2SO_4) and concentrated to give a crude solid that was recrystallized from EtOAc−hexane to give 0.78 g (3.2 mmol, 83%) of **2** as a white solid: mp 172 °C; ^{1}H NMR ($CDCl_3$) δ 1.62 (m, 4 H), 1.98 (dd, $J = 9.8$, 2.5 Hz, 2 H), 2.09−2.21 (m, 4 H), 2.32 (s, 2 H), 2.56 (t, $J = 6.8$, Hz, 1 H), 6.29 (bs, 1 H), 7.39−7.49 (m, 3 H), 7.75 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 34.9, 37.5, 43.4, 43.6, 48.8, 65.2, 126.8, 128.5, 131.2, 135.4, 166.9; IR (mull) 3351 (s), 2930 (s), 2956 (s), 1632 (s), 1579 (s), 1533 (s), 1492 (s), 1326 (m), 1293 (m) cm^{-1}; HRMS calcd 241.1467, obsd 241.1467. Anal. Calcd for $C_{16}H_{19}NO$: C, 79.63; H, 7.94; N, 5.80. Found: C, 78.35; H, 7.63; N, 5.73.

***N*-Benzoyl-*trans*-9-hydroxy-3-noradamantanamine (14) from Oxygenation of *N*-Benzoyl-3-noradamantanamine (2) with *B. bassiana*.** Bioconversion of **2** (1.80 g, 7.47 mmol) using *B. bassiana* was carried out in a 10 L tank for 3.5 days. For workup, the contents of the fermentation were centrifuged (2000 rpm, 20 min) in 1 L portions. The supernatant was extracted with 5 L of CH_2Cl_2. Similarly, the pellet was extracted with 2 L of CH_2Cl_2. The combined CH_2Cl_2 phases were dried (Na_2SO_4) and concentrated to give a crude viscous residue that was chromatographed on SiO_2. Elution was carried out with 1 L of 10% MeCN−CH_2Cl_2 followed by 1 L of 50% acetone−hexane ($R_f = 0.10$). The collected alcohol product was then twice chromatographed on SiO_2 [(1) 20% acetone−CH_2Cl_2, $R_f = 0.40$; (2) 10% acetone−CH_2Cl_2, $R_f = 0.30$] to give 1.12 g (4.37 mmol, 58%) of **14** as a white solid: mp 190−191 °C; ^{1}H NMR ($CDCl_3$) δ 1.79 (s, 1 H), 1.98 (m, 6 H), 2.14 (dd, $J = 9.5$, 3.3 Hz, 2 H), 2.30 (s, 2 H), 2.51 (m, 1 H), 3.90 (bs, 1 H), 6.34 (bs, 1 H), 7.38−7.50 (m, 3 H), 7.75 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 38.2, 43.0, 43.3, 45.0, 64.1, 72.8, 126.8, 128.5, 131.3, 135.1, 167.2; IR (mull) 3373 (s), 3258 (m), 2926 (s), 1647 (s), 1543 (s), 1491 (m), 1314 (m), 1059 (m) cm^{-1}; HRMS calcd 257.1416, obsd 257.141. Anal. Calcd for $C_{16}H_{19}NO_2$: C, 74.68; H, 7.44; N, 5.44. Found: C, 74.42; H, 7.26; N, 5.51.

Single-Crystal Structure Determination of 14: $C_{16}H_{19}O_2N$; space group P212121; cell parameters $a = 11.445(1)$ Å, $b = 12.250(1)$ Å, $c = 10.023(1)$ Å; molecular weight = 253.3006; $Z = 4$; calculated density = 1.21 g/cm^3. A clear, chunky crystal was selected and mounted on a glass fiber. The data were collected on Siemens P4 X-ray diffractometer controlled by an IBM/PC computer, at room temperature (20 °C), with graphite-monochromatized Cu Kα radiation [(Cu Kα) = 1.5405 Å]. All 1332 unique reflections were measured to a θmax of 130° for Laue group mmm; 1128 intensities were > 4σ. The XSCANS controling software (Siemens, 1994) was used for the data collection. The structure was solved by direct methods, using SHELXS version 5.0 (Siemens, 1995). The trial solution obtained 19 nonhydrogen atoms. Least-squares refinement included all nonhydrogen atomic coordinates and isotropic thermal parameters. In the final refinement cycle, with all 1332 reflections: $R = 0.164$, $S = 2.835$; Rw = 0.377; with 1128 reflections having intensities > 4σ: $R = 0.1494$.

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